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By screening cDNA libraries from primary ductal breast carcinomas with autologous patient serum, we have detected and isolated three immunoreactive cDNA clones. One of the isolated breast tumor autoantigens (*Ngp-1*), encodes a GTP-binding protein which belongs to a newly described subfamily of GTPases. Immunohistochemical analysis of tissue sections revealed that the antigen was exclusively localized in the nucleolus and nucleolar organizer regions in all cell types analyzed. The second autoantigen isolate (*Auag2*) is also a newly discovered gene product which is not expressed in transformed breast tumor epithelial cell lines, but is however detectable in breast tumors. The sequence of *AuAg2* bears a partial homology to human heparin-binding angiogenic vascular endothelial growth factor. The third potential isolate remains to be characterized, and more cDNAs are being screened with breast cancer patient serum to identify additional autoantigens.

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Janis Racevskis 8/9/95

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INTRODUCTION

Tumor growth is associated with the expression of mutated gene products, inappropriate gene expression, and the breakdown of tissue architecture, leading to the exposure and release into the peripheral circulation of sequestered antigens. Whether these circulating, mutated or newly displayed tumor-associated antigens elicit an autologous humoral immune response in the breast tumor patient is of vital interest. Isolation, identification and characterization of novel breast tumor associated autoantigens might yield new insights into the disease process, and moreover, may be developed into diagnostic screening tests and potential targets for immunotherapy.

The screening of cDNA expression libraries with autologous patient serum is a powerful technique, which has been used successfully for the identification of autoimmune disease antigens, and which we have adapted for the identification of autoantigens in cDNA libraries made from breast tumor mRNA. After screening cDNA libraries, derived from primary ductal breast carcinomas with autologous patient serum, we have detected and isolated two immunoreactive cDNA clones. Homology search sequence analysis showed that they could not be matched to any sequences present in the current Genbank database. Encouraged by our initial findings, we proposed to characterize the identified autoantigens and to construct additional cDNA libraries and screen them with autologous serum to identify and isolate additional breast tumor autoantigen cDNAs. The ultimate goals of our research project are: *1.* To isolate autoantigen clones which individually or in combination react specifically with most breast tumor patient sera and may form the basis for the development of diagnostic tests or perhaps identify potential targets for immunotherapy, and, *2.* To test the hypothesis that breast tumors result in the expression of a characteristic profile of autoantigens.

BODY

We have made considerable progress in the characterization of the first of our isolated breast tumor autoantigens, and have submitted our findings for publication (see publications, page 9).

We have named the gene encoding this autoantigen *Ngp-1*, and have determined that it encodes a GTP-binding protein. The revelation that the autoantigen is a GTP binding protein (or GTPase) is especially exciting, since GTPases are highly conserved molecular switches which control proliferation and differentiation of animal cells. GTPases are often targets of mutation and microbial toxins, and have pivotal roles in the pathogenesis of cancer and infectious diseases (1).

The complete 2.3 kb nucleotide sequence of the *Ngp-1* cDNA was found to contain an open reading frame which could encode a protein of 731 amino acids. The predicted amino acid sequence contains a high concentration of charged amino acids in the carboxy terminal quarter of the molecule, three GTP-binding protein motifs and a consensus nuclear localization signal. The arrangement and spacing of the GTP binding protein motifs indicate that *Ngp-1* belongs to a newly described subfamily of GTPases with one other known human member, the others being of prokaryotic origin (2). Except for the consensus motifs, neither nucleotide sequence, nor the predicted amino acid sequence of the *Ngp-1* cDNA showed the slightest homology to any vertebrate gene product sequence listed in the databases. It did however, show high homology to an uncharacterized partial cDNA sequence derived from rice callus. The homology with such a distant organism indicates that this gene must play a very fundamental role in cell growth. Northern blot analysis showed the 2.3 kb transcript to be ubiquitously expressed at relatively low levels in all human tissues tested, with the highest level of expression in the testes. Immunohistochemical analysis of tissue sections with affinity purified antiserum raised against a recombinant *Ngp-1* protein revealed that the antigen was exclusively localized in the nucleolus and nucleolar organizer regions in all cell types analyzed (hence our proposed name *Ngp-1:* Nucleolar G-Protein gene 1).

Since all GTPases interact with other cellular macromolecules, the next phase in our characterization of *Ngp-1* suggests itself: the identification of other gene product/products which interact with *Ngp-1* during its regulatory functions. To accomplish this we have subcloned the entire open reading frame portion of *Ngp-1* into an expression vector, and have begun to purify the full length encoded

protein. This recombinant *Ngp-1* protein will be used in binding studies with nuclear extracts to isolate target binding proteins, which will be identified by two dimensional gel electrophoresis, electrotransferred to membranes, isolated and subjected to micro amino acid sequencing for identification. If partial amino acid sequence analysis does not identify a known gene product, then degenerate oligonucleotide primers based on the amino acid sequences, will be designed for isolation of the corresponding cDNAs, and further characterization.

We have also begun to screen genomic human DNA libraries to isolate the entire *Ngp-1* gene, for eventual sequencing.

Our work on characterization of our second breast tumor autoantigen isolate (working name *Auag2*) is proceeding, and we have isolated cDNA clones encompassing most of the gene product, as judged by the size of the mRNA on Northern blots. One slight complicating factor in determining the sequence of *Auag2* is that in Northern blots, the mRNA appears as a doublet of approximately 1.7 and 1.9 kb. The isolated clones are presently being sequenced, and according to our latest data, this autoantigen is also a newly discovered gene product. Northern blot analysis indicates that *Auag2* is not expressed in transformed breast tumor epithelial cell lines, but is however detectable in breast tumor mRNA. Since breast tumors are a heterogeneous mix of many cell types, *Auag2* might be expressed by infiltrating lymphocytes or stromal fibroblasts. Localization of the autoantigen will have to await production of antiserum against recombinant *Auag2*. The available sequence bears a partial homology to human heparin-binding angiogenic vascular endothelial growth factor, a similarity with great potential relevance to breast cancer.

While work is continuing to characterize the two autoantigen isolates, we are also continuing to collect breast tumor tissue, normal breast tissue, other tumor types, patient serum and constructing more cDNA libraries for screening. Instead of screening a cDNA library with the single autologous patient serum as was done initially, we now screen with a "cocktail" mixture of breast cancer patient sera in order to increase our chances of finding autoantigens. One additional potential autoantigen clone has been isolated and will be characterized.

CONCLUSIONS

Because of the nature of our research project, we find it most productive to conduct most aspects of the work concurrently (collection of tumors and serum, construction of cDNA libraries, screening of cDNA libraries, isolation of immunoreactive clones, sequencing of clones and characterization). We think we are well on schedule with our statement of work. Because of the open ended nature of our project (we cannot predict how many immunoreactive breast tumor autoantigens we will identify), we might have to limit to what degree of detail to characterize each isolate, depending on our success rate in identifying autoantigens. Every effort will be made to characterize the isolates as fully as possible, since for newly discovered gene products, understanding their function will be a very important contribution to the understanding of breast tumor biology.

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PUBLICATIONS

Cloning of a novel nucleolar GTP-binding protein from a breast tumor. Janis Racevskis, Alyssa Dill, Richard Stockert and Susan A. Fineberg. Submitted.